Immuno-analyse et biologie spécialisée (2011) **26**, 1—5

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GENERAL REVIEWS AND PROSPECTIVE ANALYSES

Occurrence of estrogenic and pregnane X receptor specific activities in Tunisian sewage treatment plants using a panel of bioassays

Mise en évidence, à l'aide d'essais biologiques, d'activités œstrogéniques et spécifiques du récepteur X des pregnanes dans les stations d'épuration tunisiennes

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Received 28 April 2010; accepted 1st October 2010
Available online 4 December 2010

KEYWORDS
Sewage treatment plant;
Estrogen receptor;
Pregnane X receptor;
Gene reporter;
Bioluminescent cells

Summary With reporter cell lines developed in the lab and allowing the detection of specific activities, we characterized the endocrine disrupting profile of waters, particulate matter and sediments from tow Tunisian sewage treatment plants (STPs), which inputs were of domestic and touristic origins. We used bioluminescent chimeric cell lines to detect ligands of the estrogen and xenobiotic receptors. The STP waters exhibited a very high estrogenic activity whereas particulate matter and sediments showed a strong xenobiotic activity, mediated by pregnane X receptor.

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doi:10.1016/j.immbio.2010.10.003

MOTS CLÉS
Stations d'épuration ;
Récepteur des œstrogènes ;
Récepteur des prégnanes ;
Gène rapporteur ;
Cellules bioluminescentes

Résumé Grâce à la technique de culture cellulaire développée au laboratoire et permettant la détection des activités spécifiques, nous avons caractérisé la présence des activités endocriniennes dans les eaux, les matières en suspension et les sédiments de deux stations d'épuration Tunisiennes. Ces dernières sont caractérisées par des activités domestique et touristique. Nous avons utilisé des lignées cellulaires cancéreuses bioluminescentes dans la détection des ligands œstrogénique et des xéno-biotiques. Les eaux des stations d'épuration ont montré une activité œstrogénique très élevée, tandis que les particules en suspension et les sédiments ont montré une activité xéno-biotique forte spécifique du récepteur X des prégnanes.

Abbreviations

DBD	DNA-binding domain
DCC	dextran-coated charcoal-treated fetal calf serum
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulfoxide; E_2, 17β-estradiol
EC_{50}	the efficient concentration (giving 50% maximum activity)
EDC	endocrine disrupting compound
ER	estrogen receptor
ERE	estrogen-responsive element
FCS	fetal calf serum
Gal4	yeast transcription factor Gal4
LBD	ligand binding domain
Ligand eq	ligand equivalent
Luc	firefly luciferase
PAH	polycyclic aromatic hydrocarbon
PXR	pregnane X receptor
RLU	relative luminescence unit
STP	sewage treatment plant
SPE	solid phase extraction

Introduction

In recent years, many anthropogenic chemicals occurring in the environment have been shown to mimic the action of endogenous hormones. These EDCs can potentially lead to a host of adverse effects on wildlife, such as the feminization of fish, the lack of reproductive in some species, birth defects and the development of physical abnormalities [1,2]

Estrogenic EDCs include alkyl phenols, pesticides [3,4], phthalate plasticizers [5] and pharmaceutical estrogens. Pharmaceutical estrogens exhibit a high affinity towards ER, contrary to alkyl phenols, pesticides and phthalates [4]. They are hormonal contraception products like ethynylestradiol, mestranol and estradiol valerate. They are excreted by women and consequently found in waste water [6] together with the natural hormones such as 17OH-estradiol (E2) and its metabolites, estrone and estriol [7]. STPs are considered as a major source of endocrine modulator that may later contaminate the environment [3,4,8,9]. Natural or synthetic estrogens, and alkyl phenols are the predominant estrogens found in STPs [4]. Their relative concentrations vary with the type of STP-urban, industrial or agricultural [3].These contaminants are expected to be eliminated by STPs. However, they are only incompletely inactivated during treatment processes and discharged into rivers [10]. Discharges into the environment of estrogenic sewage effluent are responsible for endocrine disorders in aquatic fauna, male wild fish feminization for example [11,12], and may also have a great impact on wildlife in general.

Data concerning water contamination originating from domestic, industrial or agricultural activities suspected to contain a variety of EDCs are not sufficiently documented, in particular in Tunisia. The objectives of this study were to perform a large screening of modulators of nuclear receptors and PXR receptor in STPs.

Material and methods

Cell culture products were obtained from Life Technologies (Cergy-Pontoise, France). Luciferin was purchased from Promega (Saint-Quentin-Fallavier, France). Dioxin, E_2 and rifampicin were purchased from Sigma Chemical Co (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) at 10 mM.

Generation of stably transfected reporter cell lines

The stably transfected luciferase reporter cell lines were obtained as already described [13]. Briefly, MELN cell line was obtained by transfecting ERα positive breast cancer MCF-7 cell line with the estrogen responsive element cloned upstream of the luciferase reporter gene construct ERE-βGlob-Luc-SVNeo [14]. HGPXR reporter cell line was obtained by transfecting HG_5LN cells (derived from HeLa cells and containing a constitutively expressing luciferase downstream of Gal4RE_5-βGlob) with Gal4 (DBD) — hPXR (LBD) plasmid [15].

Cell culture conditions

HGPXR and MELN cell lines were grown in phenol red containing Dulbecco's Modified Eagle's Medium (DMEM), 1 g/l glucose, supplemented with 5% of fetal calf serum (FCS) and 1% antibiotic in a 5% CO_2 humidified atmosphere at 37 °C. Because of phenol red and FCS estrogenic activity, in vitro experiments were achieved in DMEM without phenol red, supplemented with 6% dextran-coated charcoal-treated fetal calf serum (DCC) and 1% antibiotic (penicillin/streptomycin) (test culture medium).